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Short communication

Hippocampal neurogenesis and PSA-NCAM expression following exposure to ⁵⁶Fe particles mimics that seen during aging in rats

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Abstract

Exposure to particles of high energy and charge can disrupt the neuronal systems as well as the motor and cognitive behaviors mediated by these systems in a similar fashion to that seen during the aging process. In the hippocampus, adult neurogenesis is affected both by aging and irradiation with ionizing particles. Likewise, the maturation of newly formed cells in this region as measured by PSA-NCAM expression is also altered by the aging process. The present study was designed to investigate the effects of 2.5 Gy of 1 GeV/n 56 Fe particles on neurogenesis using the nuclear proliferation marker 5-bromodeoxyuridine (BrdU and PSA-NCAM expression in the dentate gyrus of rats exposed to whole-body irradiation or simply placed in the chamber without being irradiated. All subjects (n=10) were sacrificed 28 days after the last BrdU injection (50 mg/kg X 3 days) and their brains were processed for immunohistochemistry. Results illustrate a decrease in the number of BrdU-positive cells as well as different distribution of these cells in the dentate gyrus of irradiated animals. Additionally, irradiated subjects show decreased levels of PSA-NCAM expression. These changes are consistent with those found in aged subjects indicating that heavy-particle irradiation is an adequate model for the study of aging.

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1. Introduction

Until recently, it was thought that the post-mitotic nature of neuronal cells would protect the brain from harm induced by ionizing radiation. However, research carried out by our group suggests that neurological changes occur both cellularly (Denisova, et al., 2002; Joseph et al., 2000) and behaviorally (Rabin et al., 2000; Shukitt–Hale et al., 2000, 2003) and that these effects are, in part, mediated by oxidative stress (Joseph et al., 1996). Of particular importance for its damaging effects to cells (Todd, 1983) is radiation involving high-energy particles (HZEs) such as

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signal transduction and GTPase activity in the nigrostriatal system (Joseph et al., 1992) and alterations in synaptogenesis and microneuron degeneration in the hippocampus (Philpott et al., 1985), in addition to behavioral motor (Joseph et al., 1992) and cognitive (Shukitt–Hale et al., 2000; Casadesus et al., 2004) declines occur after irradiation with various doses of HZE particles. More importantly, however, is the fact that these neuronal (Joseph et al., 1998; Anson et al., 1992) and behavioral deficits (Ingram et al., 1994; Shukitt–Hale et al., 1998) are also present in the aged rodent. Therefore, the use of HZE irradiation could provide a suitable animal model of aging.

One factor that also changes with aging (Kuhn et al., 1996; Kempermann et al., 1998) and that plays a direct role in the modulation of cognitive behavioral output (Shors et al., 2001) is the rate of hippocampal neurogenesis.

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This process encompasses the proliferation, migration and differentiation of precursor cells into functional neurons in the granular cell layer of the dentate gyrus (Gage et al., 1998; van Praag et al., 2002). In this regard, newly formed cells are born in the hiliar region and migrate to the granular cell layer (GCL) of the dentate gyrus (Kuhn et al., 1996; Gage et al., 1998). This process is decreased by age such that aged animals have fewer precursor cells in the dentate gyrus when compared to younger counterparts (Kuhn et al., 1996). In addition to changes in proliferation, migration and subsequent phenotype development are also altered by aging (Kempermann et al., 1998). These changes are possibly due to age-related declines in the expression of highly polysialylated neural cell adhesion molecule (PSA-NCAM), which is associated with the migration and maturation of immature precursor cells (Seki and Arai, 1995; Ni Dhuill et al., 1999). Therefore, although neurogenesis takes place throughout the life of an animal, decreases in plasticity associated with declines in neurogenesis and the expression of PSA-NCAM likely influence behavioral and cognitive declines seen during aging.

Since exposure to HZE particles produce 'accelerated aging' (Joseph et al., 1992, 1993) in various neuronal and behavioral parameters including hippocampally related cognitive performance (Shukitt–Hale et al., 2000, 2003; Casadesus et al., 2004), and given that newly generated neurons in the hippocampus directly modulate hippocampally related memory (Shors et al., 2001), we speculated that HZE exposure would have significant effects on hippocampal neurogenesis. Therefore, the aim of our study was to establish whether the declines in neurogenesis and PSA-NCAM seen during rodent aging could be mimicked by exposure to ⁵⁶Fe particles to validate iron-particle irradiation as a comprehensive animal model of aging.

2. Materials and methods

Ten 2-month old male Sprague–Dawley rats (240–300 g) obtained from Taconic Farms (Germantown, NY) (n=10)were individually housed and maintained at constant temperature (21 °C±1°), on a 12 h light/dark cycle and free access to food and water. All animals (n=10) were taken to and experimental animals (n=5) irradiated at the alternating generator synchrotron (AGS) at Brookhaven National Laboratory (BNL) (Upton, NY) 1 week after their arrival to the facility and 10 days prior to arrival at the HNRCA facilities (Boston, MA). The radiation procedure consisted of exposing each subject individually to wholebody irradiation with 2.5 Gy ⁵⁶Fe particles (1 GeV/n), which has been previously shown to induce neuronal and behavioral deficits (Rabin et al., 1989). All subjects were restrained in an identical fashion but because of beam time considerations control subjects were not placed in the beamline apparatus in the exposure cave. The experimental subjects were positioned so that their heads were located in the center of the beam and exposed to 2.5 Gy of 1 GeV/n 56 Fe particles at an approximate rate of 1 Gy/min which provided a fluence of 1040 particles/cm². Dose uniformity within the central 6 cm of the beam was within ± 2 –6% of the desired dose. A parallel–plate ionization chamber with Mylar windows and N_2 gas flow positioned in the beam line was used for entrance dose measurements, performed by the staff at the BNL beam facility. Once the experimental animals were declared clear of any residual radiation by the Brookhaven Radiation Department, all subjects including controls were transported to the HNRCA via overnight air.

BrdU (Sigma, St Louis, MI) was dissolved in 0.9% saline and filtered at 22 μ m. Each subject received 1 daily intraperitoneal injection (50 mg/kg) during three consecutive days starting on the day that irradiation took place. All animals were sacrificed 28 days after the last injection. The rats were sacrificed with a lethal dose of the barbiturate pentobarbital, transcranially perfused, and the brains harvested following previously established methodology (Kuhn et al., 1996). All brains were quick-frozen and sectioned coronally (40 μ m) on a cryostat.

Every sixth section was used for BrdU immunohistochemistry. Free-floating sections (40 µm thinck) stored in $1 \times PBS$ were initially transferred to 1% H_2O_2 in dH_2O for 1 h to block endogenous peroxidase. After a 1 h pretreatment (2 N HCl, 0.4% Triton-X100, 3% NHS), sections were blocked with 10% NHS for 30 min and incubated with primary anti-BrdU antibody (Mouse IgG-1:200) (Menhim-Dikinson) in solution with PBS, 3% NHS, and 0.3% Triton-X100 for 36 h at 4 °C. Subsequently, sections were incubated with secondary horse-anti-mouse-rat absorbed antibody (5 µl/ml) (Vector Labs) for 1 h at room temperature and then incubated in ABC reagent for 1 h prior to peroxidase treatment using the chromatogen diaminobenzidine (DAB) Vector Kit (Vector Labs). Alternate sections not used for BrdU immunohistochemistry, were treated with 1% H₂O₂ in dH₂O, blocked with 10% NHS for 30 min and incubated with PSA-NCAM (Mouse IgM, 1:200) primary antibody (kind donation by Dr T. Seki). All steps were preceded by 3-5 min PBS washes.

BrdU-positive cells were counted from serial coronal sections approximately 240 µm apart across the dorsal portion of the dentate gyrus (approximately 8 sections/animal). Cells were counted using a modified version of the optical fractionator method (West et al., 1991) using stereology software (Bioquant, Nashville, TN). Cells were quantified excluding those in the outermost focal plane to avoid counting cell caps. Stereological estimates were made of the total number of BrdU-positve cells located throughout the entire extend of the granular cell layer (GCL) including the subgranular zone (as defined by a two cell-body wide zone at the edge of GCL) and the hilus, as well as these regions separately, to examine migration pattern changes. Total volume was calculated by the sum of each sectioned dentate gyrus area multiplied by the thickness of the section. To minimize sampling error, sufficient fields per section

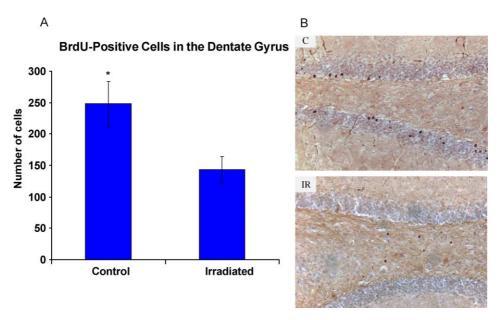


Fig. 1. (A) Mean ± SEM of total number of BrdU-positive cells in the dentate gyrus of animals irradiated with 2.5 Gy of ⁵⁶Fe high-energy radiation and control subjects. (B) BrdU-positive staining in the dentate gyrus of representative irradiated (IR) and control (C) subjects at 20X magnification.

were quantified to ensure that a minimum of 100 cells per brain were counted. Quantification of PSA-NCAM was performed following a well-established methodology for immunohistochemical quantification (Nunomura et al., 2001). Briefly, every 6th section of another series through the dorsal hippocampus was selected, and using a 5× objective a single field encompassing the entire hippocampal area, it was quantified using light microscopy (KS300, Zeiss, Munchen-Hallbergmoss, Germany) and compatible quantification software (Axiovision, Carl Zeiss Vision GmbH, Munchen-Hallbergmoss, Germany). Positive staining was expressed as optical densities that yielded the average density across the area. An area adjacent to the stained field was selected and the optical density obtained was subtracted to correct for background.

3. Results

Two-way Analysis of Variance demonstrated that the subjects irradiated with 2.5 Gy of 56 Fe radiation had a significantly lower number of total BrdU-stained cells in the dentate gyrus than did control subjects ($F_{1,8}$ =6.32, p<0.05) (Fig. 1(A) and (B)). Interestingly, when these subregions were examined separately, while the number of BrdU-positive cells in the hiliar region was similar in both groups ($F_{1,8}$ =0.05, p=0.8), the number of BrdU-positive cells in the granular cell layer was significantly higher in control subjects compared to irradiated subjects ($F_{1,8}$ =6.80, p<0.05) (Fig. 2). Furthermore, we found a significant interaction between group and brain region ($F_{1,16}$ =5.13; p<0.05) suggesting a possible inhibitory effect of 56 Fe radiation on cell migration and consequent differentiation. To further examine this later notion we

examined the expression of PSA-NCAM in the dentate gyrus and found a significant decreases in PSA-NCAM expression in irradiated when compared to control animals $(t_{1,8}=18.13, p<0.001)$ (Fig. 3(A) and (B)). Notably, a lack of correlation between declining levels of cells and PSA-NCAM expression (r=0.424, p=0.222) was observed suggesting a weak relationship between number of cells present in the GCL and PSA-NCAM immunoreactivity.

4. Discussion

The aforementioned results show that exposure to ⁵⁶Fe particles results in significant deficits in adult hippocampal neurogenesis as shown by decreased numbers of BrdU-positive cells in the dentate gyri of irradiated animals as compared to controls. These findings support previous

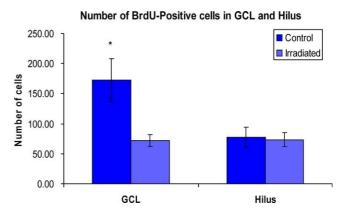


Fig. 2. Mean \pm SEM of number of BrdU-positive cells in hiliar region and GCL (including subgranular zone) of the dentate gyrus of animals irradiated with 2.5 Gy of 56 Fe high-energy radiation and control subjects.

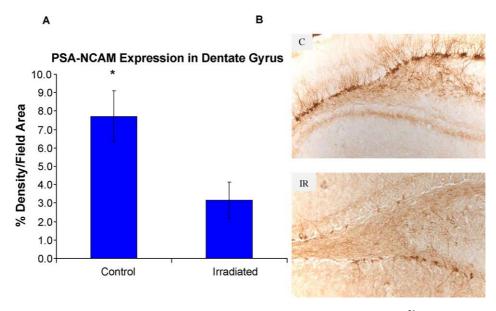


Fig. 3. (A) Mean +/- SEM of PSA-NCAM expression in the hippocampus of animals irradiated (IR) with 2.5 Gy of ⁵⁶Fe high-energy radiation and control (C) subjects as measured by % density/field area measured. (B) PSA_NCAM staining in the dentate gyrus of representative irradiated (IR) and control. (C) Subjects at 5X magnification.

research showing alterations in the proliferation of new cells in this region by other types of ionizing radiation (Madsen et al., 2003; Parent et al., 1999; Peissner et al., 1999). However, in addition to changes in proliferation, our results expand upon previous studies to illustrate unequal distribution of BrdU-positive cells between irradiated and control subjects in the various regions of the dentate gyrus. This is illustrated by the fact that while the number of BrdUpositive cells were similar in irradiated and non-irradiated groups in the hiliar region, less BrdU-postive cells were present in the granular cell layer of irradiated animals, replicating previous findings using Gamma irradiation (Monje et al., 2002) and also observed in aged subjects (Bizon et al., 2004). It has been established that precursor cells are born in the hilus and then migrate and mature in the subgranular zone where the cells acquire a phenotype (Kuhn et al., 1996). Therefore, ⁵⁶Fe heavy particle radiation likely affects the migrational machinery, rendering the cells incapable of migrating and maturing but not affecting overall neuronal viability. Such notion is supported by the decreased number of precursor cells present in the GCL of irradiated versus control animals, whereas no differences in numbers within the hilus of irradiated and control animals is observed. In this regard, although dividing and newborn neurons in the subgranular zone/GCL may be more vulnerable to irradiation than cells in the hilus, it is likely that a generalized cell death mechanism is not responsible for these differences as lower number of cells in both regions would have been observed. This notion is further supported by the observation of significant reductions in the expression of PSA-NCAM, a survival/ migration-associated marker, in the hippocampi of irradiated subjects when compared to controls. Moreover, the lack of correlation between cell number and PSA-NCAM shows that the declines in PSA-NCAM expression are likely not due to simply lower number of precursor cells in the GCL.

Finally, to date, several studies have demonstrated the capacity of various doses of ⁵⁶Fe heavy particle radiation (1.0–3.0 Gy) to produce extensive behavioral changes that mimick those seen during aging (Joseph et al., 2000) and that affect hippocampally related memory (Denisova et al., 2002; Shukitt-Hale et al., 2000, 2003; Casadesus et al., 2004) in a similar fashion. Given the strong relationship between hippocampally related performance and neurogenesis (Nilsson et al., 1999; Shors et al., 2001; van Praag, 2000), we hypothesize that changes in neurogenesis produced by ⁵⁶Fe heavy particle radiation likely play a significant role in the cognitive declines seen in the irradiated animals. Furthermore, our findings mimic alterations in neurogenesis seen in aged rodents, where both proliferation and migration are profoundly affected by this process (Kempermann et al., 1998; Kuhn et al., 1996; Seki and Arai, 1995; Seki, 2002; Bizon et al., 2004). Moreover, like during aging and neurodegenerative diseases such as AD (Nguyen et al., 2003), which is tightly associated with hippocampal degeneration and cognitive decline, irradiation is also capable of inducing microglial activation such that microglia account for 20% of BrdU-positive cells in irradiated animals (Monje et al., 2002). Therefore, it is likely that the difference in neuronal number between irradiated and control brains is even larger and it is possible that cognitive declines seen in irradiated animals are at least partially related to the activation of microglia. Likewise, inflammation and pertinent microglial activation leads to declines in hippocampal neurogensis (Ekdahl et al., 2003), mostly via reducing survival rather than proliferation. These findings suggest that microglial activation could have role in our observations regarding PSA-NCAM expression, a marker associated with survival and migration of immature neurons in the dentate gyrus, as the differential number of cells seen in the hilar region and the GCL point to a survival/ migratory event rather than a proliferation-only event. Moreover, the fact that PSA-NCAM, like neurogenesis, is upregulated by insult-induced repair (Calza et al., 2004) and that inflammation (Ekdahl et al., 2003) and irradiation (Parent et al., 1999) decrease insult-induced neurogenesis and PSA-NCAM expression futher strengthens this supposition. Future studies should use co-labeling with microglial markers to examine in depth the magnitude of microglial activation in irradiation-neurogenesis events.

Based on the above discussion, our findings illustrate that HZE particles may provide a useful model to study brain aging in rodents.

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